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REMARKS

The Invention

The present invention is directed to compositions capable of inducing an immune response to cytotoxic T cell epitopes of a full length protein in a mammal. The composition comprises an amount of *Bacillus anthracis* anthrax PA ("PA") and a full length protein bound to an APABP ("APABP") sufficient to elicit a cytotoxic T lymphocyte ("CTL") immune response. The molar ratio of PA to the full length protein bound to the APABP is greater than one and the APABP comprises at least the first 250 amino acid residues of the lethal factor of *Bacillus anthracis* and less than all of the amino acid residues of the lethal factor.

Status of the Claims

Claims 1-6 are pending in the application. Claims 1-6 stand rejected under 35 U.S.C. § 103(a).

Rejection under 35 U.S.C. § 103

Claims 1-6 stand rejected as unpatentable over Leppla *et al.*, WO 94/18332 ("Leppla *et al.*"). In making the rejection, the Examiner acknowledges that Leppla *et al.* do not teach a molar ratio of PA to full length protein bound to APABP of greater than one, but alleges that in view of the teachings of Leppla *et al.*, it would have been obvious to optimize the composition to obtain such a molar ratio. The rejection further alleges that the intended use carries no patentable weight because the claimed product is the same as an optimized product of Leppla. Applicants respectfully traverse.

To establish a *prima facie* case of obviousness, (1) there must be some suggestion or motivation, either in the reference themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior

art, not in applicant's disclosure. (*See*, M.P.E.P., § 2143, citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

All of the claim limitations must be considered and given weight when evaluating claims for obviousness (*see, e.g.*, MPEP § 2143.03). Moreover, as set forth in MPEP § 2173.05(g), a functional limitation in a claim must be evaluated and considered just like any other limitation.

The present invention is directed to compositions capable of inducing an immune response to cytotoxic T cell epitopes of a full length protein in a mammal. The composition comprises an amount of *Bacillus anthracis* anthrax PA and a full length protein bound to an APABP sufficient to elicit a CTL immune response. The molar ratio of anthrax PA to the full length protein bound to the APABP is greater than one and the APABP comprises at least the first 250 amino acid residues of the lethal factor of *Bacillus anthracis* and less than all of the amino acid residues of the lethal factor.

Thus, the present claims recite a functional limitation requiring that the claimed compositions comprise *Bacillus anthracis* anthrax PA and full length protein bound to an APABP in an amount sufficient to elicit a cytotoxic T lymphocyte ("CTL") response. As explained above, this recitation is relevant to the patentability of the presently claimed compositions.

Applicants respectfully assert that the presently claimed invention represents a separately patentable subgenus of the compositions described in Leppla *et al.* Leppla *et al.* generically discloses compositions comprising *B. anthracis* anthrax PA and ***peptides or protein fragments*** bound to an APABP and methods of using the compositions to deliver an activity to a cell (*e.g.*, a cytotoxic activity).

All of the presently pending claims require that the compositions comprising *B. anthracis* anthrax PA and ***full length protein*** bound to an APABP be an amount sufficient to ***induce a CTL response*** (*i.e.*, a MHC class I-mediated response). The claims also require that the molar ratio of PA to full length protein bound to APABP be greater than one.

In contrast to the presently claimed invention, Leppla *et al.* does not teach or suggest full length proteins bound to an APABP. In further contrast to the presently claimed

invention, Leppla *et al.* does not teach or suggest that the compositions comprising *B. anthracis* anthrax PA and peptides or protein fragments bound to an APABP be an amount sufficient to elicit a cytotoxic T lymphocyte immune response. In even further contrast to the presently claimed invention, Leppla *et al.* does not teach or suggest a molar ratio of PA to full length protein bound to APABP greater than one. Thus, at least three elements of the presently claimed compositions are absent from the disclosure of Leppla *et al.*

Moreover, without the teachings of the instant application, *i.e.*, without impermissible hindsight, one of skill in the art would have no motivation to modify the disclosure of Leppla *et al.* to make the presently claimed compositions comprising *B. anthracis* anthrax PA and full length protein bound to an APABP in an amount sufficient to induce a CTL response. Leppla *et al.* does not provide any indication as to what parameters are critical; nor does Leppla *et al.* provide direction as to the choices for making the presently claimed compositions comprising *B. anthracis* anthrax PA and full length protein bound to an APABP.

Furthermore, prior to the disclosure of the instant application, one of skill in the art would not have expected that an exogenously introduced full length protein could be processed and presented via the MHC class I pathway. As explained in Abbas *et al.*, CELLULAR AND MOLECULAR IMMUNOLOGY 133(Martin Wonsiewicz ed., W. B. Saunders 1991) (copy enclosed), "endogenously synthesized antigens end up associated with class I MHC and exogenously synthesized and endocytosed antigens end up associated with class II MHC."

In contrast to Leppla *et al.*, the presently claimed invention provides the first evidence that a bacterial toxin system (*i.e.*, anthrax toxin) can be used to introduce a full length protein into the cytosol for processing via the MHC class I pathway and presentation by MHC class I molecules to CTLs. More particularly, the present invention is the first demonstration that a full length protein, fused to LF, and translocated into a cell by anthrax toxin is processed by the cytosolic MHC class I pathway and presented by MHC class I molecules to CTL's. For example, as set forth in the specification at page 3, lines 4-11 and page 18, lines 20-28, the processing of *B. anthracis* anthrax PA and full length protein bound to an APABP via the MHC class I pathway was confirmed by the use of the specific proteasome inhibitor, lactacystin. The presence of lactacystin significantly decreases the CTL response to target cells contacted with

the *B. anthracis* anthrax PA and full length protein bound to an APABP, thus confirming that processing of peptide epitopes from the full length protein occurs via the MHC class I pathway.

Thus, the present invention is a selection invention directed to a separately patentable subgenus of compositions comprising *B. anthracis* anthrax PA and full length protein bound to an APABP in an amount sufficient to induce a CTL response.

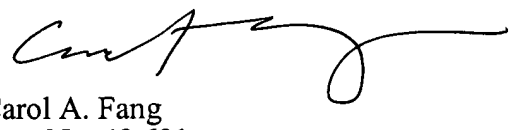
Since, Leppla *et al.* does not disclose all of the elements of the presently claimed invention and one of skill in the art would not have been motivated to modify the disclosure of Leppla *et al.*, Applicants respectfully submit that a *prima facie* case of obviousness has not been established. Applicants therefore respectfully request withdrawal of the rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged.

If the Examiner believes a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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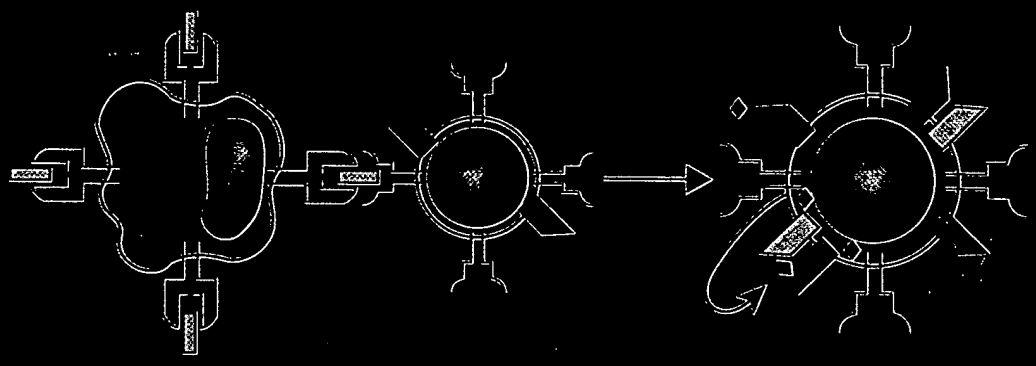

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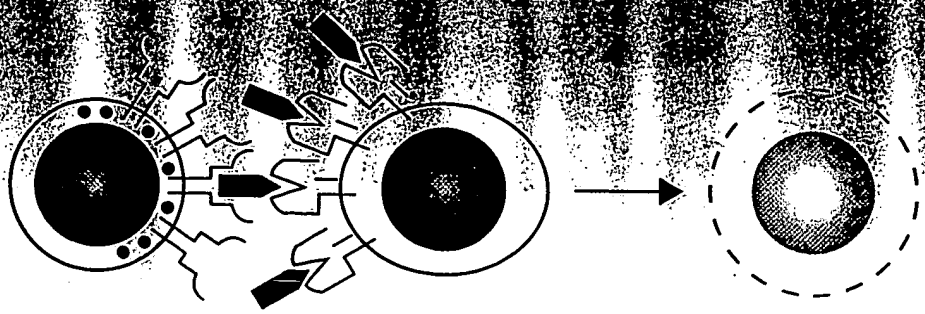

CELLULAR AND MOLECULAR IMMUNOLOGY

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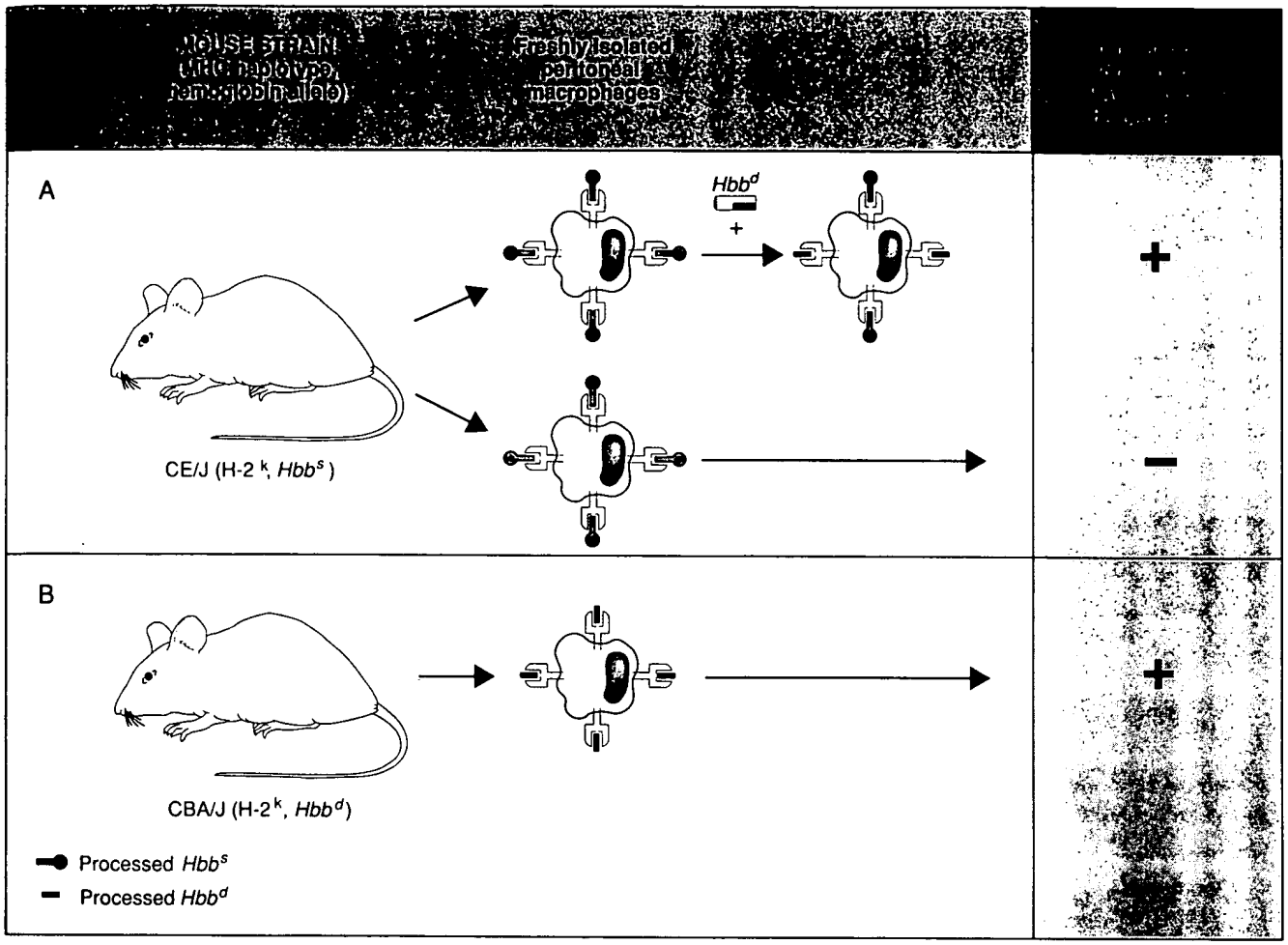

FIGURE 6-9. Macrophages present self hemoglobin in association with self MHC molecules in vivo. *Macrophages from* Hbb^s*-expressing mice will stimulate* Hbb^d*-specific I-A^k-restricted T cells only if the antigen,* Hbb^d*, is added (A). However, freshly isolated macrophages from* Hbb^d*-expressing mice stimulate the same T cells without any requirement for exogenous antigen (B). Therefore, the freshly isolated macrophages from* Hbb^d*-expressing mice must bear processed* Hbb^d *on their surface.*

Class I MHC-Associated Antigen Presentation

As we have mentioned previously, CD8$^+$ T cells, most of which are CTLs, recognize class I MHC-associated foreign protein antigens. Furthermore, CD8$^+$ T cells usually recognize protein antigens that are synthesized within APCs and subsequently expressed on the surface in association with class I MHC molecules. Examples of endogenously synthesized foreign proteins are viral proteins and tumor antigens. CTLs are the principal immunologic defense mechanisms against viruses and may be important in the immune destruction of tumors.

In vitro analyses of the binding of foreign peptides to class I MHC molecules indicate that it is essentially similar to peptide-class II binding. Each class I MHC molecule has a single binding cleft that accommodates peptides that are 10 to 20 amino acids long. The affinity of class I-peptide association is in the order of 10^{-6} M. Different peptides can bind to the same site in a class I MHC molecule and compete with one another for presentation. Any one peptide can also bind to class I and class II MHC molecules, and there are no structural motifs that confer specificity for class I or class II MHC association on an antigenic peptide. Whether a particular antigen will be presented by an APC in association with class I or class II MHC molecules is apparently determined by the intracellular compartmentalization of the protein. Antigens that are synthesized endogenously within the APCs generally traverse different compartments from those antigens that are endocytosed from the extracellular environment. This is supported by several studies:

1. If a viral protein, such as influenza nucleoprotein, or a protein like ovalbumin is added in soluble form to a cell that expresses class I and class II MHC molecules, the antigen is internalized, processed, and presented only in association with class II MHC molecules. Such exogenously added antigens will be recognized by class II-restricted, antigen-specific CD4$^+$ T cells but will not sensitize the APC to lysis by CD8$^+$ cells. On the other hand, if the gene encoding the viral

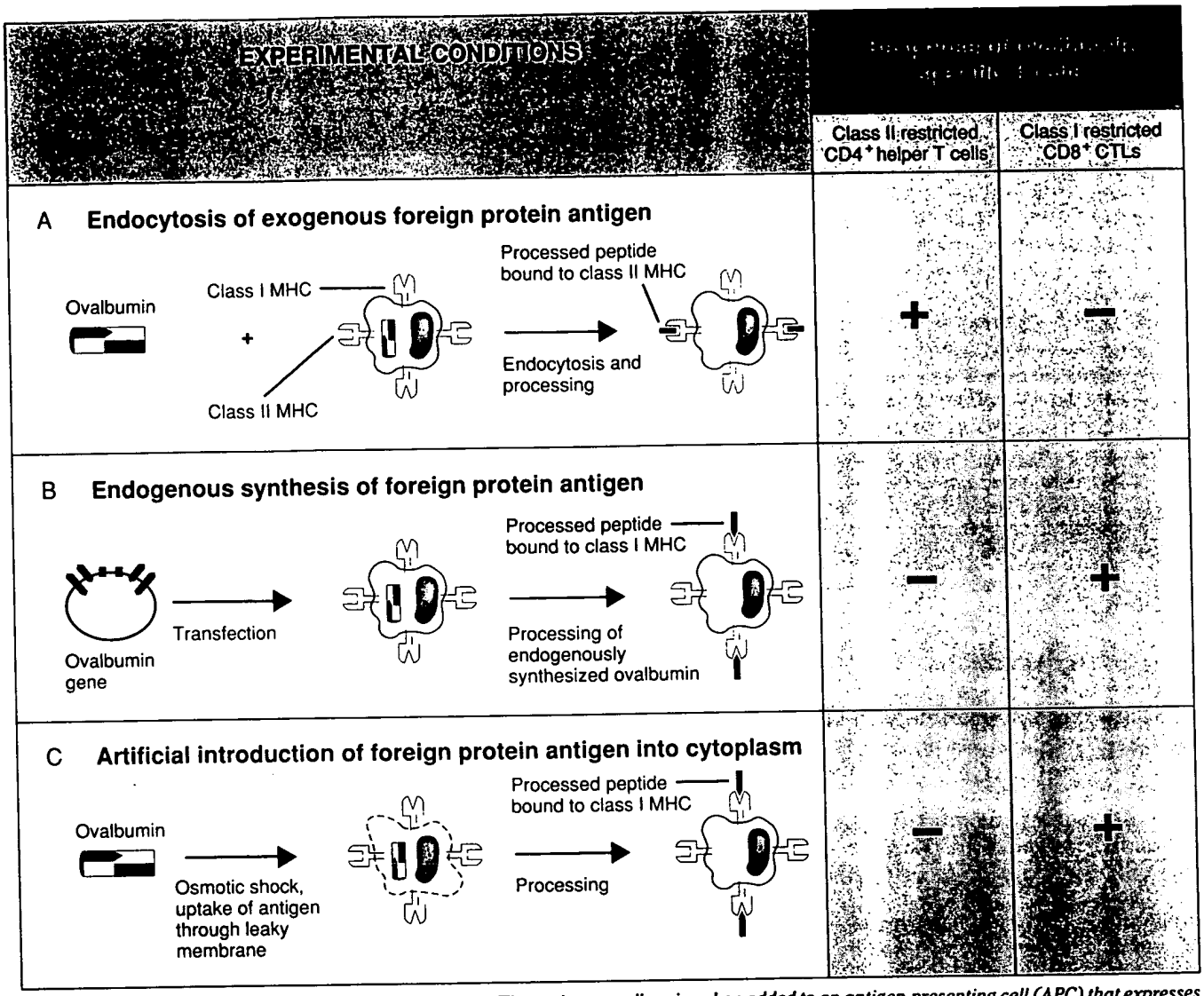

FIGURE 6-10. Presentation of exogenous and endogenous antigens. *The antigen, ovalbumin, when added to an antigen-presenting cell (APC) that expresses class I and class II MHC molecules, is presented only in association with class II* (A). *The same ovalbumin synthesized intracellularly as a result of transfection of its gene* (B) *or introduced into the cytoplasm by osmotic shock* (C) *is presented in association with class I MHC molecules. The measured response of class II restricted helper T cells is cytokine secretion and the measured response of class I-restricted cytolytic T lymphocytes (CTLs) is killing of the APCs.*

protein or ovalbumin is transfected into the APCs so that the antigen is synthesized endogenously, the cell becomes sensitive to lysis by specific class I-restricted $CD8^+$ cells (Fig. 6-10). APCs that express these transfected gene products do not stimulate $CD4^+$ T cells. Intracellular localization rather than endogenous synthesis may be the critical factor determining the class I association of antigenic peptides. For instance, if an antigen is introduced into the cytoplasm of a cell by making the plasma membrane transiently permeable to macromolecules, the antigen is subsequently processed and peptides associate only with class I MHC molecules (Fig. 6-10). This further supports the concept that the traffic patterns of intracellular and endocytosed proteins are different.

2. Presentation of some endogenously synthesized viral antigens to $CD8^+$ CTLs cannot be inhibited by chloroquine, whereas presentation of exogenously added viral proteins to $CD4^+$ T cells is chloroquine-sensitive. This suggests that processing of endogenously produced antigens may not occur in acidic endosomes.

3. *Class I-restricted presentation of endogenously synthesized viral antigens requires association of antigen with newly synthesized class I MHC molecules in the endoplasmic reticulum (ER).* This has been demonstrated in two ways. First, a pharmacologic inhibitor of protein transport out of the ER, called Brefeldin A, can block the post-translational processing and transport of all newly synthesized proteins including self class I and class II MHC and foreign viral proteins. This drug treatment inhibits the class I-restricted presentation of endogenously synthesized viral protein, more than the class II-restricted presentation of

exogenously encountered proteins. Second, the adenovirus E19 protein specifically binds to and prevents transport of class I MHC molecules out of the ER. The ability of E19 to block nascent class I transport correlates with its ability to block class I-restricted antigen presentation.

Thus, the association of antigens with class I versus class II MHC molecules is due to the trafficking of the antigens through different intracellular compartments (Fig. 6-11). In most cases, the commitment to one or another traffic pattern is determined by where the antigen comes from; endogenously synthesized antigens end up associated with class I MHC and exogenously synthesized and endocytosed antigens end up associated with class II MHC. There are exceptions, however, when endogenously synthesized proteins do end up being presented in association with class II MHC molecules.

Many unanswered questions remain about the cell biology of class I-restricted antigen presentation. The site at which antigens are processed into peptides before association with class I MHC molecules is not known, nor is it understood how the processed peptides get into the rough ER, where nascent class I MHC molecules are being synthesized. There is some evidence that the ER itself may contain proteolytic enzymes that could generate immunogenic peptides. Since both class I and class II MHC molecules are produced in the rough ER and both have a natural affinity for peptides, there must also be some mechanism that prevents peptides from binding to class II molecules in the ER. This may be accomplished by the class II-associated invariant or γ chains that may interfere with the peptide-binding clefts. Class I MHC molecules do not have invariant chains when they are synthesized and are therefore free to bind peptides produced within the cell. This may be one reason why

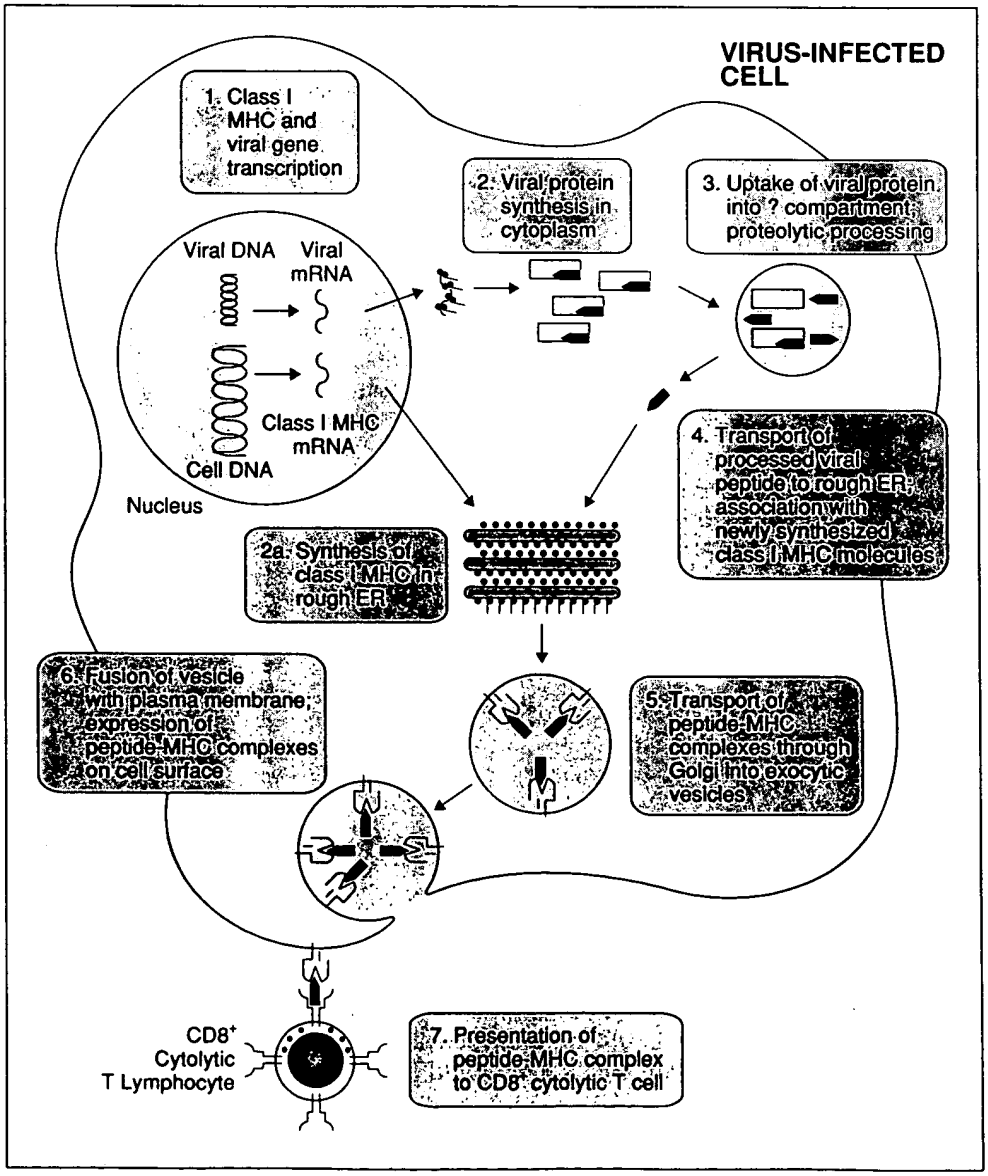

FIGURE 6-11. *Pathway of class I MHC-restricted presentation of an endogenously synthesized (e.g., viral) antigen.*

endogenously synthesized antigens become class I-associated. As the class II MHC molecules are being transported in vesicles to the cell surface, they shed the invariant chain. At this stage, the vesicles containing MHC molecules may intersect with endosomes containing internalized, processed antigens. Since the binding clefts of the class I molecules are already occupied by peptides, the endocytosed and processed antigens bind to the only available MHC molecules, which are now the class II molecules.

PHYSIOLOGIC SIGNIFICANCE OF MHC-ASSOCIATED ANTIGEN PRESENTATION

So far we have discussed the specificity of $CD4^+$ and $CD8^+$ T lymphocytes for MHC-associated foreign protein antigens and the mechanisms by which complexes of peptides and MHC molecules are produced. It is important to also consider the physiologic implications of this rather unusual specificity of T cells. The central role of MHC molecules in T cell antigen recognition influences the immunogenicity of different protein antigens and the response patterns of the T cells.

Immunogenicity of Protein Antigens

Our present understanding of the role of MHC genes in determining the ability of protein antigens to induce specific immunity is limited largely to exogenous antigens. MHC molecules may determine the immunogenicity of protein antigens in two related ways:

1. *The immunodominant epitopes of complex proteins are often the peptides that bind most avidly to MHC molecules.* If an individual is immunized with a multideterminant protein antigen, in many instances the majority of the responding T cells are specific for one or a few linear amino acid sequences of the antigen. These are called the "immunodominant" determinants or epitopes. For instance, in H-2^k mice immunized with HEL, more than half the HEL-specific T cells are specific for the epitope formed by residues 46 to 61 of HEL in association with the I-A^k but not the I-E^k molecule. This is because HEL(46-61) binds to I-A^k better than do other HEL peptides and does not bind to I-E^k. However, it is not yet known exactly which structural features of a peptide determine immunodominance. The question is an important one because an understanding of these features may permit the efficient manipulation of the immune system with synthetic peptides. An obvious application of such knowledge is the design of vaccines. For example, a protein encoded by a viral gene could be analyzed for the presence of amino acid sequences that would form a typical immunodominant secondary structure capable of binding to MHC molecules with high affinity. Vaccines composed of synthetic peptides mimicking this region of the protein theoretically would be effective in eliciting T cell responses against the viral peptide expressed on an infected cell, thereby establishing protective immunity against the virus.

2. *The expression of particular class II MHC alleles in an individual determines the ability of that individual to respond to particular antigens.* The phenomenon of immune response (Ir) gene-controlled immune responsiveness was mentioned in Chapter 5. We now know that Ir genes that control antibody responses are class II MHC genes. They influence immune responsiveness in part because various allelic class II MHC molecules differ in their ability to bind different antigenic peptides and, therefore, to stimulate specific helper T cells. For instance, H-2^k mice are re-

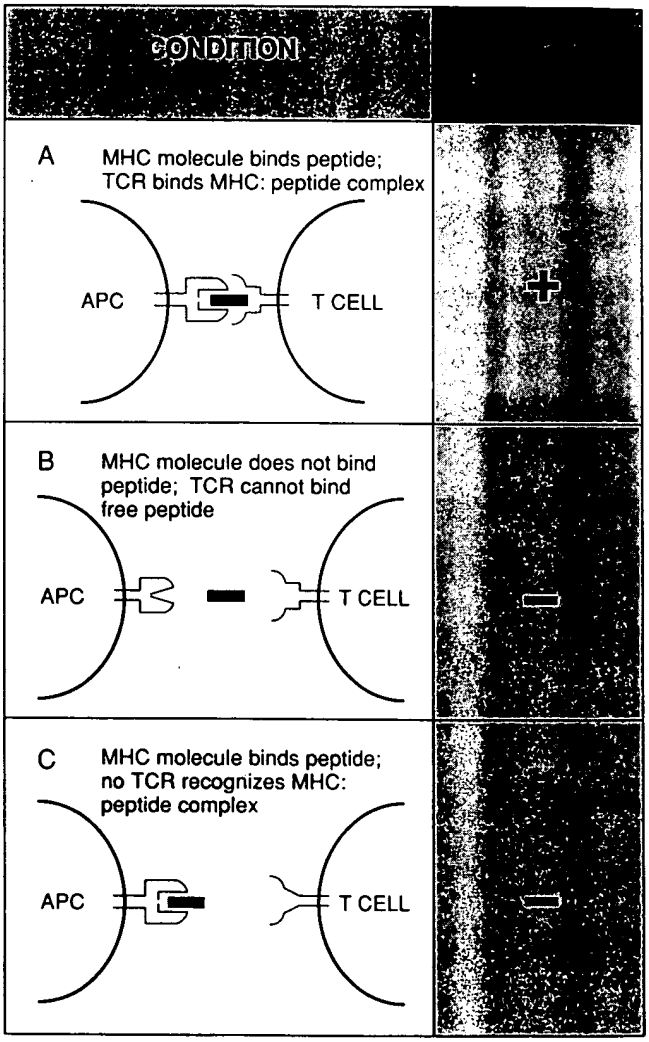

FIGURE 6-12. Mechanisms of MHC-linked immune response (Ir) gene function. *Antigen presentation and T cell activation occur when an individual expresses MHC molecules that can bind peptides derived from the processed antigen and T cells are present that specifically recognize complexes of these MHC molecules with the peptides* (A). *If an individual does not inherit genes encoding MHC molecules that can bind the peptides, no T cell response occurs* (B). *Alternatively, if no T cells are present which recognize the MHC molecules as self, no T cell response occurs* (C). *The development of self-restricted T cells is discussed in Chapter 8.*